NEW APPROACHES FOR THE DESIGN OF MULTI-REGION HYBRIDIZATION ASSAYS (MHAS) FOR HIV-1 GENOTYPING AND A SECOND-GENERATION ASSAY FOR SOUTHEAST ASIA

Kijak GH, Tovanabutra S, Sanders-Buell E, Watanaveeradej V, Kim R, de Souza M, Brown AE, Birx DL and McCutchan FE

Background: The Thai HIV-1 epidemic is rapidly evolving, with the circulation of subtype B, CRF01_AE and their recombinants. Subtype C and C-containing strains, found in neighboring countries, are expected to impact the HIV-1 genetic diversity in Thailand. A Multi-region Hybridization Assay (MHAbce) (Watanaveeradej et al., XIV Intl AIDS Conf 2002) developed earlier, was revised to accommodate the changing molecular picture. Here we present a new approach for MHA development and a second-generation MHAbce for Southeast Asia.

Methods: Nucleic acids were extracted from plasma or PBMCs. First-round amplicons were generated by RT-PCR or PCR. 384-well plate real-time PCR with subtype-specific fluorescent TaqMan probes was used to assess the genotype at 8 genomic regions; the specific amplicon production was monitored by using Sybr-Green (SG) and melting curves (MC). The samples' genotype was deduced from the patterns of probe reactivity.

Results: A novel approach was used for the development of probes and primers for real-time PCR. Candidate probes were first synthesized as unlabeled oligonucleotides (ONTs) and were evaluated by MC with SG in their hybridization properties towards synthetic ONTs representing Southeast Asian strains. Those showing the highest specificity and sensitivity were synthesized as labeled probes. Different primer-pair combinations were tested by SG-based real-time PCR with MC, and those showing optimum sensitivity, efficiency and minimum primer-dimer generation were selected. The real-time PCRs were then assessed using the selected primers and labeled probes on a panel of 12 prototypic strains and the assay performed with high specificity and sensitivity. A field test on a larger sample set is underway.

Conclusions: New design approaches for real-time PCR HIV-1 genotyping permitted rapid re-design of the MHAbce to accommodate the increasing diversity of the Thai epidemic. Large-scale screening of strains is now possible to support vaccine trials.

XV International AIDS Conference. Bangkok, Thailand. 11-16 July 2004.

PHASE III TRIAL OF HIV PRIME-BOOST VACCINE COMBINATION IN THAILAND

Rerks-Ngarm S, Pittisutthithum P, Nitayaphan S, Brown AE, Kitayaporn D, Gurunathan S, Heyward W and McNeil J

Background: The world's first community-based, phase III HIV vaccine trial began in Thailand in late 2003. This is being carried out through the infrastructure of the Ministry of Public Health, augmented by Mahidol University and supported by the Armed Forces Research Institute of Medical Sciences.